IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ORIGINAL

In re application of:

ZAUDERER *et al.*

Appl. No. 09/987,456

Filed: November 14, 2001

For: ***In Vitro* Methods of Producing and Identifying Immunoglobulin Molecles in Eukaryotic Cells**

Confirmation No.: 6770

Art Unit: 1639

Examiner: Epperson, J.D.

Atty. Docket: 1821.0070004/EJH/T-M

Declaration Under 37 C.F.R. § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, Dr. Maurice Zauderer, residing at 44 Woodland Road, Pittsford, New York 14534, declare and state as follows:

1. I am the co-founder of Vaccinex, Inc., and have held the positions of President and Chief Executive Officer since April 6, 2001. I am also a co-inventor of the captioned patent application.

2. A current *curriculum vitae* is appended hereto as Exhibit B1.

3. I received my Ph.D. degree in cell biology from the Massachusetts Institute of Technology in 1972. From 1971 to 1975, I conducted postdoctoral research at various research institutions including the Albert Einstein College of Medicine in New York, and the National Institute for Medical Research in London. I was an Assistant Professor in the

Department of Biological Sciences at Columbia University from 1976 to 1983, and from 1984 to 2000, I was an Associate Professor in the Cancer Center and Department of Microbiology and Immunology at the University of Rochester. As shown on my attached *curriculum vitae*, I have also held various other academic positions, as well as participating in many professional activities and publishing numerous peer-reviewed articles in the field of immunology. Based on my education and experience, I am an expert in immunology and cell biology.

4. I have reviewed the above-identified patent application ("patent application"), the final Office Action dated January 27, 2005; PCT Publication No. WO 93/01296 to Rowlands *et al.* ("Rowlands"); PCT Publication No. WO 93/01296 to Zauderer ("Zauderer"); and Waterhouse *et al.*, *Nucleic Acids Res. 21*:2265-2266 (1993) (collectively, "the cited references"). I have also reviewed the pending claims of the patent application.

5. The invention claimed in the patent application relates to the field of immunology. More particularly, the invention relates to the art or field of methods of identifying, producing, and/or expressing immunoglobulins in eukaryotic cells. In my opinion, a person of ordinary skill in the art of immunology would have a Ph.D. degree in a field related to immunology or cell biology.

6. The claimed invention is directed to a method of selecting polynucleotides which encode an antigen-specific human immunoglobulin molecule by introducing into a population of mammalian host cells first and second libraries of polynucleotides encoding

immunoglobulin subunit polypeptides, wherein the libraries are constructed in vaccinia virus vectors, and the construction does not entail traditional homologous recombination.

7. It is my understanding, as explained to me by Vaccinex, Inc.'s patent attorneys, that the question of obviousness involves whether one of ordinary skill in the art would have been motivated by the prior art to make and use the claimed invention, and whether the claimed invention could have been practiced with a reasonable expectation of success. Using this standard of obviousness, it is my view that the disclosure of Rowlands in view of Zauderer and Waterhouse would not have motivated one of ordinary skill to combine these references, and would not have provided one of ordinary skill with any reasonable expectation of success in making and using the present invention. It is also my understanding, as explained to me by Vaccinex's patent attorneys, that a *prima facie* showing of obviousness can be overcome by a showing of objective indicia of non-obviousness of the claimed invention, such as unexpected results, a long-felt and unsolved need, commercial success, and failed attempts by others. It is my opinion that the response to the claimed invention in the field shows that there was a long-felt and unsolved need for it.

8. At the time of the present invention, there were two primary technologies used in the field of immunology to produce, identify, and select antigen-specific fully human antibodies from a large repertoire of possible immunoglobulins: transgenic animals expressing exogenous immunoglobulin genes and phage display. The transgenic animal technology, besides being time consuming and costly, is limited by the fact that antibodies to human proteins that are produced in a transgenic animal--for example, a mouse--having a

homolog of that protein may be biased toward epitopes that are different between the human and the animal host. However, these may not be the optimal target epitopes. This is a general problem (known as "tolerance") because almost all important human genes have a murine homolog and, on average, these are 90% homologous at the protein level. In some cases, where there is a very high degree of homology, no useful antibodies can be selected in immunoglobulin transgenic animals.

9. Phage display technology is also prone to numerous drawbacks. For example, it is not possible to generate full size human antibodies using phage display. Rather, phage display is limited to producing immunoglobulin fragments which are expressed as a fusion with a phage protein, with immunoglobulin fragments displayed on the surface of a phage particle. Based on my communications with others in the field, one of the major problems that is associated with using phage display to select antigen-specific immunoglobulins, is that, once the antigen-specific variable region is isolated from the phage and expressed as an IgG molecule, it often no longer recognizes the target antigen. That is, antigen-specificity was only achieved when the variable region was expressed in a prokaryotic host, incorporated onto a phage particle. Expression on phage particles results in another difficulty in screening for antibodies specific for membrane-associated proteins that are difficult to purify (e.g., G-protein coupled receptors), and which must therefore be screened as whole cells or cell fragments, because phage particles have non-specific interactions with mammalian cells, and thereby interfere in the antibody screening process. Furthermore, antibodies produced in prokaryotic cells do not undergo normal eukaryotic post-translational modification and assembly, and often lose specificity as a result of

incorrect folding or conformation in the abnormal physiological environment of the bacterial cell. *See, e.g.,* Specification Paragraph [0412] of the captioned application. The present invention overcomes these problems.

10. The Office Action states that it would have been obvious to one of skill in the art to combine Rowlands, Zauderer, Waterhouse, and Marasco to arrive at the methods of the claimed invention. In my opinion, one of skill in the art would not have found the claimed invention obvious in light of Rowlands, Zauderer, and Waterhouse. Detailed support for my opinion is set forth below.

11. Rowlands describes the use of known protein expression techniques using vaccinia virus vectors to express a single recombinant antibody. The expression of the single recombinant antibody was performed by introducing known, previously cloned sequences into vaccinia virus vectors by traditional homologous recombination. While Rowlands mentions that the vectors may be used to express a human antibody, the only example provided is of a humanized antibody, Campath-1H.

12. Zauderer describes introducing into eukaryotic host cells a single library of vaccinia virus expression vectors constructed from tumor-cell derived DNA, RNA, or cDNA to identify a single polypeptide of interest.

13. Waterhouse describes a method of using a Cre-*lox* site-specific recombination system in *E. coli* bacteria to pair an immunoglobulin light chain variable region fragment

and an immunoglobulin heavy chain variable region fragment carried in bacteriophage vectors so that they can be simultaneously co-selected. The expressed proteins are fusions of antibody fragments with a phage protein. Waterhouse suggests that the method "should allow the creation of extremely large combinatorial repertoires, " Waterhouse at 2266, col. 1.

14. In my view, the cited references do not teach all of the limitations of the claimed invention. For example, the references do not teach the introduction of ***two*** libraries of expression vectors into eukaryotic host cells to select an antigen-specific immunoglobulin.

15. It is also my opinion that one of skill in the art would not have been motivated to combine the cited references to introduce two separate expression libraries of immunoglobulin heavy and light chains into eukaryotic host cells to select a previously unknown antigen-specific immunoglobulin. One of ordinary skill would not have thought to combine Rowlands and Zauderer because, although they both describe the use of vaccinia virus vectors, Rowlands does not suggest the introduction of expression libraries into eukaryotic cells at all, and Zauderer does not suggest the introduction into host cells of two expression libraries that separately encode immunoglobulin heavy and light chains. Furthermore, one of ordinary skill in the art would not have combined Waterhouse with Rowlands and Zauderer because, while Waterhouse suggests providing separate repertoires of light and heavy chain antibody fragments, the suggestion is in the context of a method for improving phage display techniques. As discussed above, phage display is used to produce

libraries of human antibody fragments in prokaryotic host cells using bacteriophage as the vectors, where at least one of the antibody fragments is expressed as a fusion protein with a phage surface protein. There is no suggestion in Waterhouse that the types of improvements contemplated therein could be used in a eukaryotic system; nor would one of ordinary skill in the art consider them as features that could be expanded for use in eukaryotic systems. Given the above, one of ordinary skill in the art would not have had a reasonable expectation of success in combining Rowlands, Zauderer, and Waterhouse to arrive at the present invention.

16. It is also my opinion that there was a long-felt need for the present invention in the field of immunology and antibody selection. As discussed above, prior to the present invention, fully human antibodies were produced, identified, and selected by using transgenic animal technology, or human antibody fragments were produced, identified, and selected as bacteriophage fusion proteins by phage display techniques. The transgenic animal technology suffers from the drawbacks of time, expense, and the issue of tolerance that tends to produce antibodies that do not have useful activity. Phage display technology, developed to overcome the problems associated with the transgenic animal technology, requires expression of antibody fragments only, as fusion proteins with phage surface proteins, without the benefit of eukaryotic post-translational modification and assembly, and often results in antibodies that, once removed from the context of the fusion protein, lose the ability to specifically recognize target antigen. The present invention overcomes the drawbacks associated with both of these technologies.

17. By way of another example, antibody fragments generated by phage display can only be screened for antigen binding activity, not for functionality. For instance, antibody glycosylation is important to antibody function. Antibody fragments that are produced in a prokaryotic system as in phage display do not undergo eukaryotic glycosylation. Therefore, antibody fragments isolated by phage display, although they may bind antigen, cannot be tested, for example, for effector function, which depends on having a constant region and the presence of key glycosylation residues, without isolating the V region and recloning it as full size immunoglobulin.

18. Evidence of the long-felt need for the present invention can be seen by the number of strategic alliances that have been established between companies that would like to produce antigen-specific human monoclonal antibodies for therapeutic and diagnostic purposes and Vaccinex, Inc., exclusive licensee of the present invention. Attached hereto as Exhibits B2-B4 are copies of press releases announcing collaborations between Vaccinex, Inc., and the following companies: OPi, Lonza Biologics, and Biocon Limited. The fact that these companies are interested in pairing with Vaccinex, Inc. to use the technology of the present invention indicates that there is a desire and interest to use these methods for developing human antibodies because the prior art methods were not suitable for the needs of the marketplace.

20. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Maurice Zauderer

Maurice Zauderer, Ph.D.

Date: July 20, 2005